Root Exudates as Integral Part of Belowground Plant Defence

Ulrike Baetz

Abstract Root exudates comprise a heterogeneous group of compounds that display various effects on soilborne organisms, including stimulation, attraction, but also repellence and inhibition. Therefore, root-secreted chemicals can assist belowground plant defence through direct and/or indirect mechanisms. Direct defence strategies exploited by roots include the secretion of phytochemicals with antimicrobial, insecticide, or nematicide properties. In contrast, other root exudates recruit or influence beneficial organisms to serve as biological weapons against plant aggressors, a mechanism termed indirect plant defence. Since rhizosecretion fundamentally shapes the composition of soil-inhabiting organisms and contributes to plant survival, the quality and quantity of defence root exudates are tightly controlled. Various environmental and endogenous factors can stimulate the release of phytochemicals that exhibit precisely targeted bioactivities. On the molecular level, several primary active transport proteins have been demonstrated to affect the composition of defence root exudates in the rhizosphere. In this chapter, we will focus our attention on direct and indirect defence strategies mediated by root exudates. In addition, we will shed light on regulatory mechanisms of defence-related root exudation that prevent belowground disease and ensure optimal plant performance.

1 Introduction

Plants interact with a multitude of soilborne organisms in complex biological and ecological processes in the narrow zone surrounding the root system, termed the rhizosphere. These beneficial, antagonistic, or neutral interactions have a profound effect on plant health and survival and shape the soil microbiome.

Within the rhizosphere, roots are constantly exposed to biotic stressors, ranging from plant disease-causing pathogens such as bacteria, fungi, and oomycetes to nematodes and insects. Although being sessile organisms anchored to the soil, plants are not just passive victims of these antagonistic microbes and invertebrates

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that occur in the vicinity of roots. In fact, roots are equipped with an arsenal of defence compounds that can be released into the rhizosphere to counteract plant attackers (Baetz and Martinoia 2014). However, the significance of root exudates as a direct or indirect belowground protection has long been underestimated, presumably due to literally being out of sight.

Secreted substances can be of low or high molecular weight. Low-molecular-weight root exudates include a variety of defence secondary metabolites such as flavonoids, glucosinolates, and terpenoids. Protective high-molecular-weight compounds such as antimicrobial proteins and secreted extracellular DNA also contribute to the local belowground resistance. The tremendous metabolic diversity of root exudates has been progressively elucidated in the past decade through the identification and characterization of numerous novel constitutively secreted and inducible compounds and previously undescribed classes of defence molecules. Equally, genes and biosynthetic pathways involved in the production of these phytochemicals have been gradually deciphered. A deepened knowledge of phytochemical properties, their composition in the rhizosphere, and their impact on soil-inhabiting organisms is crucial to understand the diverse nature of root-exudate-mediated defence mechanisms that protect plants against pathogens and invaders. It has been demonstrated that some root exudates exhibit antibacterial, antifungal, nematicide, or insecticide properties that directly assist the plant in coping with antagonistic organisms. Other root exudates are released from damaged roots to attract natural enemies of the attackers (such as carnivorous nematodes) to indirectly protect plants. Another highly sophisticated indirect defence strategy of plants is to outsource defence compound production. On that purpose, root exudates attract beneficial microorganisms that release secondary metabolites such as antibiotics with an antagonistic effect on the root-attacking pathogen.

In this chapter, we will compile the roles of root exudates in various direct and indirect, targeted belowground defence processes that protect plants against soilborne diseases. In addition, we will discuss regulation mechanisms of root exudation, e.g., inducible substance production and controlled secretion, that collectively make root-exudate-mediated belowground plant defence a highly efficient process.

2 Root Exudation as a Direct Defence Strategy Against Detrimental Soilborne Organisms

In the rhizosphere, roots face relentless harmful attack through the presence of plant disease-causing pathogens (e.g., bacteria, fungi, and oomycetes), as well as root-damaging animals (in particular nematodes and insects). In the following, we will illustrate with selected examples how aggressors are being repelled, inhibited, or killed by certain root-secreted phytochemicals in order to confer direct defence against belowground plant diseases.

2.1 Bacteria

The bacterial community in the soil is diverse in its composition, ranging from beneficial plant growth-promoting bacteria to bacteria that infect roots and exhibit harmful effects. Plant-derived molecules can act as chemical signals that stimulate or repress microbes. Thereby, root exudates fundamentally drive the selection of bacteria inhabiting the rhizosphere. Shifts in root-exudate blends, as observed in an *Arabidopsis* (*Arabidopsis thaliana*) mutant impaired in root exudation, elicited significant compositional alterations in bacteria that colonize the rhizosphere (Badri et al. 2009). Furthermore, it has been recently reported that merely the application of root exudates collected from *Arabidopsis* modulated the overall native bacterial community in the soil, even in the absence of the plant (Badri et al. 2013). Conversely, the chemical profile of root-secreted molecules is largely dependent on distinct bacterial members present in the vicinity of roots. For instance, the formation and release of the antimicrobial monoterpene 1,8-cineole were induced upon compatible interactions between *Arabidopsis* roots and the bacterial pathogen *Pseudomonas syringae* DC3000 (Steeghs et al. 2004; Kalemba et al. 2002). In another study, *Arabidopsis* roots that were exposed to *P. syringae* secreted significantly higher amounts of defence-related proteins, whereas the incompatible interaction with a bacterial symbiont did not induce the secretion of these protective proteins (De-la-Peña et al. 2008).

A phytochemical known to feature direct antibacterial activity particularly against *Pseudomonas aeruginosa* is rosmarinic acid (RA) (Bais et al. 2002). This multifunctional caffeic acid ester is produced in hairy root cultures of sweet basil (*Ocimum basilicum* L.) and exuded in response to pathogen attack. However, the compound is absent from exudates of unchallenged root cultures (Bais et al. 2002). *Arabidopsis* root exudates that were supplemented with exogenous RA prior infection with pathogenic *P. aeruginosa* strains highly reduced pathogenicity under in vitro and in vivo conditions (Walker et al. 2004). Without supplementation, *Arabidopsis* roots displayed a high level of susceptibility to *P. aeruginosa* resulting in mortality. Similarly, the induction of RA secretion by sweet basil roots before infection conferred resistance to *P. aeruginosa* (Walker et al. 2004). Hence, host plants can deliberately release antibacterial molecules into the rhizosphere that directly counteract root colonization of pathogenic bacteria and plant mortality.

2.2 Fungi and Oomycetes

Tremendous yield losses result from fungal root invasion every year, emphasizing the necessity to study the cross talk between plants and fungi and to elucidate root exudates that confer direct disease resistance. In fact, oomycetes are phylogenetically distinct organisms but show high physiological and morphological similarities to fungi. Therefore, fungi and oomycetes will be both covered in this section.

A potent root-secreted antimicrobial compound that is implemented into defence mechanisms against oomycete pathogens is the pea (*Pisum sativum*) isoflavonoid pisatin (Cannesan et al. 2011). Once pea roots were challenged with the oomycete *Aphanomyces euteiches*, the biosynthesis and release of pisatin into the rhizosphere were induced (Cannesan et al. 2011). Interestingly, the inoculation also had a stimulatory effect on border cell production of pea. Border cells are metabolically active cells at the root periphery that originate and detach from the root cap meristem (Stubbs et al. 2004; Vicré et al. 2005; Driouich et al. 2007). They assist the growing root tip during the mechanical penetration of the soil by decreasing frictional resistance at the root–soil interface (Driouich et al. 2007). In addition, antimicrobial molecules in the rhizosphere largely derive from cap and border cells (Hawes et al. 2012; Griffin et al. 1976; Odell et al. 2008), revealing a link between the *A. euteiches* induced formation of border cells and the increased pisatin exudation (Cannesan et al. 2011). The exposure of pea root tips encompassing border cells to exogenous pisatin, in turn, led to the upregulation of border cell production in vitro (Curlango-Rivera et al. 2010). Hence, border cells and their exudates account for a local protective shield that is strengthened in response to pathogen invasion (Cannesan et al. 2011; Hawes et al. 2012; Curlango-Rivera et al. 2010). Because a correlation was observed between border cell separation and the induction of protein secretion, Wen et al. (2007) proteolytically degraded the root cap secretome during inoculation with the pea-pathogenic fungus *N. haematococca*. The researcher demonstrated that protease treatment increased the percentage of infected root tips significantly, providing evidence that root-secreted defence proteins from border cells contribute fundamentally to the resistance of pea roots to fungal infection (Wen et al. 2007). Detailed proteome analysis of root exudates of several plant species confirmed the secretion of antimicrobial enzymes and demonstrated dynamic compositional changes during development and upon pathogenic interactions (De-la-Peña et al. 2010; Shinano et al. 2011; Liao et al. 2012; Ma et al. 2010; De-la-Peña et al. 2008; Wen et al. 2007). Unexpectedly, besides defence-related proteins, also the DNA-binding protein histone H4 was detected in border cell exudates of pea (Wen et al. 2007). Histone-linked extracellular DNA (exDNA) is thought to have a critical role in defence against microbial pathogens in mammals (von Köckritz-Blickwede and Nizet 2009; Brinkmann et al. 2004; Medina 2009). In plants, exDNA linked to histone proteins has been found to be exuded from root border cells and suggested to be a component of direct belowground defence against fungal invasion (Wen et al. 2009). Similar to proteolytic solubilization of exuded proteins, nuclease treatment of pea root tips resulted in enhanced susceptibility to fungal infection by *N. haematococca* (Wen et al. 2009). However, the distinct mechanism of how exDNA inhibits pathogen infection awaits elucidation (Hawes et al. 2011, 2012). In addition to protective proteins and exDNA, also low-molecular-weight antimicrobial root exudates are proved direct chemical weapons against soilborne diseases of fungal origin. For instance, the phenolic compound *t*-cinnamic acid potently protects barley (*Hordeum vulgare*) against the soilborne fungus *Fusarium graminearum* (Lanoue et al. 2010a, b).

2.3 Nematodes

Nematodes are wormlike eukaryotic invertebrates that consume bacteria, fungi, or other nematodes, and some can parasitize plants. Intense research on root-secreted compounds uncovered attractants that influence the chemotaxis response of beneficial nematodes or assist pathogenic nematodes in host recognition. Other phytochemicals have been found to exhibit nematode-antagonistic properties (Reynolds et al. 2011; Curtis 2008; Hiltpold and Turlings 2012). Lilley et al. (2011) investigated the potency of a root-exuded direct defence compound against nematodes. The researchers showed that the root cap targeted expression and release of a nematode-repellent chemodisruptive peptide in *Arabidopsis thaliana* reduced the establishment of the beet cyst nematode *Heterodera schachtii* (Lilley et al. 2011). In line with this, it was found that transgenic *Solanum tuberosum* (potato) that secreted this repellent peptide from their roots suppressed parasitism by the potato cyst nematode *Globodera pallida* (Lilley et al. 2011; Liu et al. 2005). In another study, a genetic approach was used to broaden the resistance of soybean (*Glycine max*) against nematodes. An *Arabidopsis* gene that modulates synthesis of the antimicrobial camalexin and other defence-related responses was ectopically overexpressed in roots of soybean (Youssef et al. 2013), resulting in enhanced resistance to the parasitic soybean cyst nematode (*Heterodera glycines*) and the root-knot nematode (*Meloidogyne incognita*). Lauric acid, a naturally occurring, highly abundant root exudate from crown daisy (*Chrysanthemum coronarium*) also limited parasitic damage by decreasing the number of *M. incognita* and suppressing nematode infection (Dong et al. 2014). Likewise, total root-cap exudates from various legumes showed the ability to repel root-knot nematodes in sand assays (Zhao et al. 2000). In summary, root exudates can have direct nematotoxic or repelling effects to ensure protection of the roots. However, in contrast to compounds with antimicrobial activity, examples for nematicide root exudates remain limited.

2.4 Insects

As plants cannot escape belowground insects and root feeding causes tremendous tissue damage, roots employ elegant defence strategies to counteract herbivory. For instance, the semi-volatile diterpene hydrocarbon, rhizathalene A, is constitutively produced and released by noninfected *Arabidopsis* roots (Vaughan et al. 2013). Plants that are deficient in rhizathalene A production were found to be less resistant to herbivory by the fungus gnat (*Bradysia* spp.) and suffered considerable removal of peripheral tissue at larval feeding sites. In this study it was comprehensively shown that rhizathalene A is a local antiherbivore metabolite that is implicated in the direct belowground defence against insect herbivory (Vaughan et al. 2013). The monoterpene 1,8-cineole is another volatile compound that exhibits defence activity. It is released from *Arabidopsis thaliana* roots upon compatible interaction with the herbivore *Diuraphis noxia* (Steeghs et al. 2004). However, little is known about root-released volatiles and other root exudates with insecticidal properties that directly defend plants against root-feeding arthropods. Nevertheless, as discussed in the following, belowground volatile compounds and their protective role were extensively studied as an indirect defence trait.

3 Root Exudates Are a Tool to Establish Indirect Plant Defence

Direct defence via root exudation is an effective mean of plants to deal with the constant exposure to pathogenic microbes and invertebrates in the rhizosphere. Besides, by root exudation plants can influence the behavior of phytobeneficial soil organisms to serve defensive roles during belowground diseases. For instance, the orientation of rhizospheric nematodes that are predators of insect aggressors can be altered by root-released signals, thereby indirectly conferring resistance to the roots against herbivory (Rasmann et al. 2005). Furthermore, some rhizobacteria species are known for their production of toxic compounds targeting plant pathogens, a process that has been hypothesized to be regulated by root exudates upon infection (Jousset et al. 2011; Haas and Défago 2005). A scenario in which plants recruit defence-assisting organisms to counteract pathogen attack is considered indirect belowground plant defence. This tripartite interaction is mediated by root exudates.

3.1 Recruitment of "Natural Soldiers" by Root Exudates

The concept of indirect defence and the corresponding plant-released signaling compounds has been examined thoroughly in the aboveground terrestrial environment. Leaves emit a complex battery of volatile organic compounds to communicate with their environment and attract predators. Intriguingly, when attacked by belowground herbivores, plants can also attract soilborne mobile predators such as entomopathogenic nematodes (EPNs). In fact, EPNs are plant protagonists but obligate parasites that kill insect hosts. The pivotal role of root-emitted volatile compounds that act as efficient cues to direct natural enemies such as EPNs specifically to the sites where potential hosts are damaging roots has become increasingly evident in the last years (Hiltpold and Turlings 2008; Hiltpold et al. 2011). The best studied example of a volatile signal that mediates belowground indirect plant defence is the maize (*Zea mays* L.) sesquiterpene olefin (*E*)-β-caryophyllene (EβC) (Rasmann et al. 2005). EβC is completely absent in healthy maize roots but emitted upon feeding by voracious larvae of the Western corn rootworm (WCR), *Diabrotica virgifera virgifera*. Herbivore attack induces the expression of the *terpene synthase 23* (*tps23*) gene, which is involved in the biosynthesis of EβC (Capra et al. 2014; Köllner et al. 2008). The released volatile signal strongly attracts the EPN *Heterorhabditis megidis*, a natural enemy of root-feeding herbivores that assists maize defence by killing WCR larvae (Rasmann et al. 2005).

WCR is a severe pest causing tremendous yield losses particularly on maize roots (Miller et al. 2005). Exploiting naturally produced indirect defence compounds against WCR could provide an effective biological control strategy for crop protection. Degenhardt et al. (2009) aimed at promoting plant attractiveness to natural enemies of WCR larvae by genetically introducing EβC emission in maize varieties that are not capable of synthesizing the sesquiterpene due to a lack of *tps23* transcript. On that purpose, a non-emitting maize line was transformed with an (*E*)-β-caryophyllene synthase from oregano (*Origanum vulgare*), resulting in a constitutive emission of EβC (Degenhardt et al. 2009). In field experiments, transformed plants attracted EPNs more efficiently and consequently suffered less root feeding by WCR larvae compared to non-emitting maize plants. In a subsequent study, it has been demonstrated that a constitutive emission of the volatile signal generated also physiological costs such as compromised seed germination, plant growth, and yield (Robert et al. 2013). This negative effect on plant fitness was possibly due to an increased attraction of herbivores, including aboveground pests. Ali et al. (2010, 2012) similarly exercised caution when investigating the complex effects of belowground volatiles on indirect plant defence. Citrus roots release volatile compounds such as pregeijerene (1,5-dimethylcyclodeca-1,5,7-triene) in response to feeding by the larvae of the root weevil, *Diaprepes abbreviatus* (Ali et al. 2010, 2012). The herbivore-induced volatile emission recruited a naturally occurring EPN (*Steinernema diaprepesi*), resulting in an increase of root weevil mortality and, hence, the control of herbivore infestation (Ali et al. 2010, 2012). Yet, further research uncovered that besides the recruitment of beneficial nematodes, herbivore-induced volatiles also allowed more efficient host localization by phytopathogenic nematodes (Ali et al. 2011). Collectively these studies illustrate clearly that consequences evoked by the manipulation of belowground volatile emission should be carefully assessed on multitrophic levels and under field conditions in order to understand their specificity and minimize detrimental physiological or ecological effects for plants or nontarget organisms.

Besides targeting volatile emission, another elegant approach to enhance the effectiveness of indirect plant defence is selective breeding of natural enemies for increased responsiveness to a volatile host signal in order to obtain a more efficient natural finding and killing of pests. Hiltpold et al. (2010a) aimed at improving the attraction of *Heterorhabditis bacteriophora*, one of the most virulent nematodes against WCR larvae, toward EβC (Hiltpold et al. 2010a). After few generations of selection, the researchers isolated an *H. bacteriophora* strain that was significantly more attracted to the EβC source than the original strain. Consistently, in field experiments WCR populations that attacked EβC-emitting maize roots were more effectively reduced by the selected strain compared to the original strain. Importantly, control experiments showed that this artificial selection for the responsiveness trait of *H. bacteriophora* toward the volatile signal has not considerably altered other essential properties for controlling WCR populations such as the infectiveness of *H. bacteriophora* (Hiltpold et al. 2010a, b).

Taken together, the research shows that plants can recruit natural enemies of their soilborne aggressors through root-released volatiles to indirectly defend the root system. Thoroughly exploited manipulation of indirect plant defence has a great potential as an alternative method to traditional broad-spectrum pesticides in controlling root pests in agroecosystems.

3.2 Root Exudates Can Stimulate the Antimicrobial Potency of Phytobeneficial Microbes

Besides attracting natural predators of their enemies, plants have established dialogues with beneficial root-colonizing bacteria to protect roots against the attack of deleterious rhizosphere microorganisms. Defence-assisting microbes belong to so-called plant growth-promoting rhizobacteria (PGPR) (Compant et al. 2005). PGPR primarily stimulate plant growth by, e.g., the production of phytohormones or the enhancement of plant nutrition (Vacheron et al. 2013). In contrast, defence-assisting PGPR can improve plant health either directly by repelling plant aggressors with the production of antibiotics or indirectly by eliciting induced systemic resistance in host plants (Compant et al. 2005; Haas and Défago 2005; Doornbos et al. 2012). However, to date only few studies addressed the role and the chemical nature of plant-derived exudates in the suppression of soilborne diseases via direct bacterial antagonism (Neal et al. 2012; Neal and Ton 2013; Santos et al. 2014; Jousset et al. 2011; Haas and Défago 2005; Notz et al. 2001; Baehler et al. 2005; de Werra et al. 2008, 2011). Jousset et al. (2011) made an elaborate experiment providing compelling evidence that plants are able to influence the metabolism of beneficial rhizosphere-colonizing bacteria through root exudates as part of the indirect belowground plant defence against pathogens. In order to prevent physical contact between the microorganisms, barley plants were grown in a split-root system in which one part of the roots was challenged by the pathogenic oomycete *Pythium ultimum*. The other side was inoculated with the biocontrol bacterium *Pseudomonas fluorescens* CHA0, a PGPR known to assist crop plant defence by producing antifungal chemicals against pathogenic fungi (Haas and Défago 2005). This separation system allowed the investigation of alterations of bacterial gene expression patterns that are induced by pathogens but mediated by systemic signaling of plants and root exudation (Fig. 1). The researchers found that the expression of the bacterial *phlA* gene was considerably stimulated following pathogen infection at the other side of the root (Jousset et al. 2011). The expression of this gene reflects the production of the antifungal metabolite 2,4-diacetylphloroglucinol (DAPG), a key component of the biocontrol activity of root-associated bacteria acting in disease suppression (Notz et al. 2001; Bangera

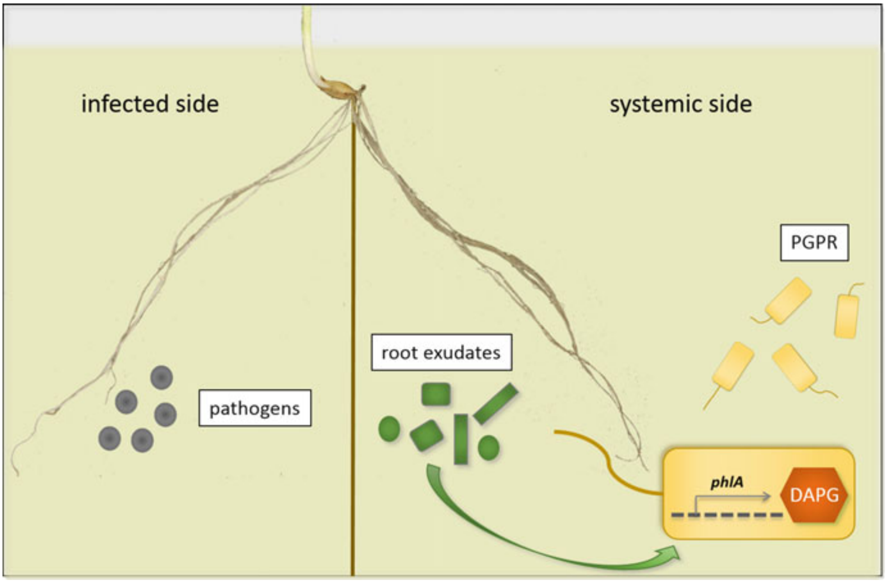

Fig. 1 Relevance of systemic plant signaling and root exudation in a tripartite interaction that confers indirect plant defence. To investigate pathogen-induced but plant-mediated modulation of bacterial gene expression and antifungal activity, Jousset et al. (2011) grew barley in a split-root system (Jousset et al. 2011). One part of the root (infected side) was challenged with the pathogen *Pythium ultimum*, whereas the other part of the root (systemic side) was exposed to the beneficial plant growth-promoting rhizobacterium (PGPR) *Pseudomonas fluorescens* CHA0. Without physical contact but through systemic plant signaling, pathogen attack induced compositional changes in root exudates on the systemic side. These changes, in turn, stimulated bacterial *phlA* expression. The transcript levels of this gene directly correlate with the production of the antifungal compound 2,4-diacetylphloroglucinol (DAPG)

and Thomashow 1999; de Souza et al. 2003). Interestingly, also the composition of exudates from the systemic side at which roots were inoculated with *P. fluorescens* changed in response to the presence of the pathogen *Pythium ultimum* at the other side of the root system (Fig. 1), uncovering candidates of signaling root exudates that provoke changes in antifungal gene expression of beneficial bacteria (Jousset et al. 2011). In summary, first insights have been gained on how antifungal activities of rhizobacteria can be adjusted by root exudates to provide service of indirect defence against plant pathogens. It will be of interest to further explore this tripartite interaction and investigate how and which plant-derived compounds are released under pathogen pressure and subsequently modulate rapidly the activity of plant growth-promoting rhizobacteria.

4 Root Exudation: A Tightly Regulated and Highly Efficient Process

Root exudation enormously impacts plants as well as the rhizosphere habitat. Firstly, photosynthetically fixed carbon is a valuable resource for plants. Since direct and indirect defence root exudates are a significant carbon cost, sensible and deliberate use is of importance to avoid excessive consumption but guarantee efficient plant defence. Secondly, root-exudate blends need to be carefully assembled, since the rhizosphere is composed of a diverse variety of inhabitants such as beneficial and pathogenic organisms that can be differentially affected by certain phytochemicals. On the purpose of accurate plant defence and limited damage to other rhizosphere members, plants have established several strategies to optimize root exudation, including elicitation-induced compound production, tightly regulated export processes, and multiple beneficial compound activities, which will be discussed in the following sections.

4.1 Constitutive Versus Induced Exudation of Phytochemicals

Plants are constantly exposed to soilborne antagonists. To form a protective buffer zone around roots, certain defence root exudates are constitutively released into the rhizosphere. For instance, rhizathalene A, an antifeedant involved in direct plant defence, is synthesized and secreted from *Arabidopsis* roots even in the absence of root-feeding insects (Vaughan et al. 2013). Plants secrete a wide array of other defence molecules before pathogen elicitation (Kato-Noguchi et al. 2008; Toyomasu et al. 2008; Wen et al. 2009; De-la-Peña et al. 2010; Shinano et al. 2011; Chaparro et al. 2013; Badri et al. 2010; Liao et al. 2012; Ma et al. 2010; McCully et al. 2008; Dong et al. 2014). Besides a constitutive root exudation, the biosynthesis, accumulation, and secretion of certain defence molecules can be induced in the presence of aggressors in the rhizosphere. The phenolic compound *t*-cinnamic acid is an antifungal exudate of barley roots (Lanoue et al. 2010a, b). Upon attack of a fungal pathogen, labeling experiments demonstrated the de novo biosynthesis and secretion of this aromatic defence metabolite into the rhizosphere. Another example is rosmarinic acid, which is constitutively produced in root tissue but exclusively released into the rhizosphere in response to root infection (Bais et al. 2002). These studies illustrate that the profile of root exudates is not just diverse in its composition but also strikingly dynamic, to adjust the identity and amount of defence compounds toward necessity in heterogeneous environments.

4.2 *Stimuli That Control Defence Root Exudation*

As discussed above, the belowground attack by antagonistic organisms can induce the release of a multitude of defence compounds into the rhizosphere. Astonishingly, upon aboveground attack, intraplant chemical signals can be relayed to influence root exudation (Bezemer and van Dam 2005; Robert et al. 2012; Pangesti et al. 2013). Secretion of L-malic acid from *Arabidopsis* roots is stimulated by infection with the bacterial foliar pathogen *Pseudomonas syringae* pv. *tomato* Pst DC3000 (Rudrappa et al. 2008; Lakshmanan et al. 2012). Elevated levels of malic acid in the rhizosphere in turn recruit the beneficial *Bacillus subtilis* FB17 and promote rhizobacterial colonization to enhance plant defence (Rudrappa et al. 2008; Lakshmanan et al. 2012).

Under laboratory conditions, the rhizosecretion process can be elicited also by exogenous application of biotic stress-related signaling molecules such as salicylic acid, nitric oxide, or methyl jasmonate (Badri et al. 2008b; Badri and Vivanco 2009; Ruiz-May et al. 2009; Schreiner et al. 2011). Likewise, an ectopic expression of the oomycetal elicitor β-cryptogein in hairy roots of *Coleus blumei* mimics pathogen attack resulting in an enhanced level of secreted antimicrobial rosmarinic acid in the external culture medium (Vuković et al. 2013). Recently it has been reported that the presence of phytobeneficial bacteria can enhance root volatile emission required for indirect plant defence (Santos et al. 2014). Root colonization with *Azospirillum brasilense* induced higher release of (*E*)-β-caryophyllene from maize roots. Furthermore, larvae of the South American corn rootworm, *Diabrotica speciosa*, gained less weight when feeding on rhizobacterium-inoculated roots (Santos et al. 2014).

Besides exogenous stimuli that influence the release of compounds implemented in direct and indirect plant defence, root exudation is also under the control of endogenous genetic programs such as the developmental stage of the plant. In maize benzoxazinoids form a class of defence molecules (Ahmad et al. 2011) that are released during the emergence of lateral and crown roots when the plant is locally and temporally more susceptible (Park et al. 2004). Hence, benzoxazinoid secretion presents a genetically regulated, protective process that alleviates damage at local sites or during discrete developmental stages when infection is more deleterious for the plant. In accordance, the peak of defence-related protein exudation into the rhizosphere can be observed just before flowering (De-la-Peña et al. 2010). Toward later stages of the *Arabidopsis* life cycle, also the level of putatively antimicrobial phenolic compounds increases in the root-exudate profile (Chaparro et al. 2013). Again, the recruitment of phytobeneficial microbes that indirectly prevent root infection through the production of antibacterial compounds is dependent on the growth stage of the plant (Picard et al. 2000, 2004).

Taken together, these studies exemplify that the secretion of defence compounds into the rhizosphere is a tightly controlled, spatiotemporal dynamic process that is regulated by various endogenous and exogenous factors.

4.3 The Role of Transport Proteins in Root Exudation

Root exudation is in part mediated by diffusion, channels, and vesicle transport. However, a substantial proportion of root exudates is also secreted actively by transport proteins. First indirect evidence of a primary and secondary active secretion process of plant-derived molecules across the root plasma membrane came from comprehensive pharmacological studies. The use of various inhibitors revealed that the secretion of some root-derived phytochemicals was dependent on ATP hydrolysis (Loyola-Vargas et al. 2007), indicating that active transport systems such as ATP-binding cassette (ABC) transporters might be involved in the release of constituents of the root phytochemical cocktail into the rhizosphere. ABC-type proteins constitute a large family of transporters that are involved in mediating the transport of a wide array of organic substances (Yazaki et al. 2008, 2009; Kang et al. 2011). More than 120 genes in the *Arabidopsis thaliana* genome encode for ABC transporter proteins, and some of these genes exhibit strikingly high expression in root cells, raising the potential for their involvement in rhizosecretion processes (Badri et al. 2008a). Subsequent studies in which root-exudate (Badri et al. 2008a, 2009) and microbial (Badri et al. 2009) compositions of ABC transporter mutants differed significantly from those of corresponding wild-type plants confirmed the essential role of ABC proteins in root exudation. In addition, these studies revealed that multiple ABC transporters can release the same substrate and that a discrete ABC transporter can have low substrate specificity and export multiple structurally and functionally unrelated substances (Fig. 2a). The role of *At*ABCG37/*At*PDR9 in mediating the rhizosecretion of not only auxinic compounds (Ito and Gray 2006; Ruzicka et al. 2010) but also of phenolics as an iron acquisition strategy (Rodríguez-Celma et al. 2013; Fourcroy et al. 2014) supports this observation. Likewise, *At*ABCG36/*At*PDR8 is suggested to export cadmium (Kim et al. 2007) as well as indole-3-butyric acid (Strader and Bartel 2009) into the rhizosphere.

To date, few ABC transport proteins were proposed to be implemented in the export and accumulation of phytochemicals that confer resistance against soilborne diseases. For example, silencing *Nt*ABCG5/*Nt*PDR5 from tobacco (*Nicotiana tabacum*) improved larval performance of the herbivore *Manduca sexta* but also increased slightly the susceptibility to the soilborne fungus *Fusarium oxysporum*, suggesting a role of this transport protein in defence inter alia through root exudation (Bienert et al. 2012). More evidently, the transporter *Np*PDR1 of *Nicotiana plumbaginifolia* was shown to be involved in belowground plant defence against pathogen invasion (Bultreys et al. 2009; Stukkens et al. 2005). Silencing the ABC transporter accounted for enhanced sensitivity of roots and petals toward several fungal and oomycetal pathogens, possibly due to diminished secretion of antimicrobial compounds such as the diterpene sclareol (Bultreys et al. 2009; Stukkens et al. 2005; Jasiński et al. 2001). Besides these obvious connections between a transporter, its substrate, and a direct effect on the rhizosphere microbiome, further research on ABC proteins implemented in root exudation

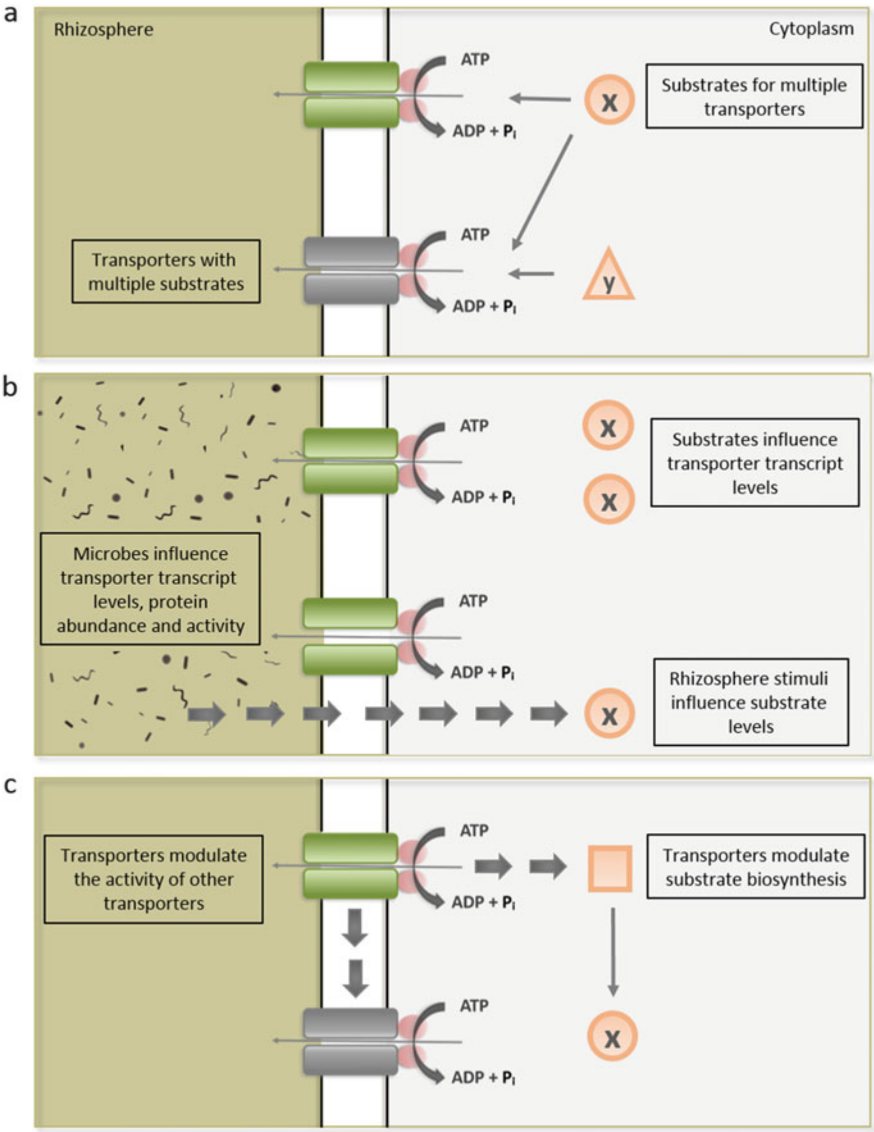

Fig. 2 ABC proteins are complex transport systems that modulate root exudation. (**a**) Some ABC proteins transport multiple substrates. Equally, some compounds can be a substrate of several transporters. (**b**) Transporter transcript levels, protein abundance, and activity can be dependent on substrate availability, elicitors, and microbial presence. In addition, rhizosphere stimuli can influence substrate production. (**c**) ABC transporters can pleiotropically modulate cell physiology, e.g., by influencing substrate biosynthesis or the activity of other transporters

uncovered a complex role for transport systems in determining the composition of root exudates (Fig. 2). Certain ABC transporter genes are subject of intense transcriptional regulation. The expression of *Nt*PDR1 from tobacco can be modified by microbial elicitation and positively correlates with export rates of antipathogenic diterpenes into the extracellular medium (Crouzet et al. 2013; Sasabe et al. 2002). In line with this, the transcriptional regulation of ABC transporters in response to their substrates has been reported (e.g., Kretzschmar et al. 2012). The level of an external phytochemical can be dependent on the transport protein abundance but also on the substrate availability. For instance, nitrogen deficiency can elicit the increased production of the flavonoid signaling molecule genistein resulting in its secretion from soybean roots to initiate rhizobium symbiosis (Sugiyama et al. 2008). Interestingly, the transport machinery involved in genistein export is constitutively active, regardless of the nitrogen availability (Sugiyama et al. 2007) (Fig. 2b). Yet, other ABC transporters themselves feature regulatory functions influencing biosynthesis and exudation of defence phytochemicals. *Medicago truncatula* roots silenced for *Mt*ABCG10, a close homolog of *Nt*PDR1 (Sasabe et al. 2002; Crouzet et al. 2013), were rapidly infected by *Fusarium oxysporum* (Banasiak et al. 2013). The silencing resulted also in a reduction of the antimicrobial medicarpin as well as its precursors in root tissue and exudates. Thus, during belowground biotic stress response, *Mt*ABCG10 supposedly modulates isoflavonoid levels associated with the de novo biosynthesis of defence compounds (Banasiak et al. 2013). Another persuasive study showed that the root-exudate profile of the *Arabidopsis* mutant *abcg30* exhibits a decreased secretion of certain compounds, whereas other exudates accumulated to higher levels in the mutant plant rhizosphere (Badri et al. 2009). These findings suggest that ABC transporters have a sophisticated role in mediating substrate export into the rhizosphere but also in directly or indirectly modifying other physiological processes such as the biosynthesis of secondary metabolites and/or the expression of other transporters involved in root exudation (Fig. 2c).

Besides ABC transporters, members of the multidrug and toxic compound extrusion (MATE) protein family have been demonstrated to actively transport secondary metabolites across plant membranes (Yazaki et al. 2008). A MATE transporter in the stele of rice roots was found to facilitate efflux of phenolic compounds into the xylem (Ishimaru et al. 2011). It has been speculated that similar transporters might be responsible for the secretion of antimicrobial compounds into the soil. A crucial root exudation process that has been shown to be mediated by MATE proteins is the release of citrate into the rhizosphere (Furukawa et al. 2007; Fujii et al. 2012; Magalhaes et al. 2007; Liu et al. 2009; Maron et al. 2010). Since citrate is a carbon source for many microorganisms, this exudation may have a vital impact on microbial soil communities. However, to our knowledge, no evidence has been provided for an implementation of MATE transport proteins in direct or indirect belowground plant defence.

Taken together, active transport systems largely influence the composition of root exudates and can dynamically adjust the quality and quantity of certain phytochemicals in response to changes in microbial rhizosphere communities.

Identification and investigation of transporter proteins implemented in regulated rhizosecretion processes are fundamental to understand belowground direct and indirect plant defence.

4.4 One Phytochemical- Additive Defence Functions

In the previous sections, we demonstrated that the release of defence-related root exudates is inducible, how this induction can be elicited, and that regulated secretion is mediated on the molecular level by transport proteins. In this section, we will highlight that single root exudates can target multiple rhizosphere organisms and may elicit dissimilar responses. Belowground plant defence becomes highly efficient if different exudate bioactivities are appropriately fine-tuned to allow an opposite effect on plant mutualists and antagonists.

Some root-secreted defence compounds affect a highly specific spectrum of rhizosphere organisms. For instance, the legume root-exudate canavanine exhibits cytotoxic properties against many soil bacteria but initiates the detoxification machinery of rhizobia, accounting for their resistance to canavanine (Cai et al. 2009). In *Arabidopsis*, resistance to *Phytophthora capsici* relies on the production of the antimicrobial camalexin (Wang et al. 2013); however, this defence compound does not confer resistance to the oomycetes pathogen *Phytophthora cinnamomi* (Rookes et al. 2008). Notably, this high target specificity of root exudates can be partially explained by variations in the tolerance to specific defence molecules based on the efficiency of active detoxification and efflux processes between different microbes (Cai et al. 2009; Bouarab et al. 2002).

Other root exudates have a broader recipient spectrum and affect various rhizosphere organisms, including beneficial and pathogenic members (Badri et al. 2013). This can be exemplified by the different effects of green pea (*Pisum sativa*) root exudates on the behavior of beneficial and plant-parasitic nematodes (Hiltpold et al. 2015). Low concentrations of root exudates induced the loss of mobility and a state of reversible quiescence in antagonistic nematodes, protecting the roots against infection. In sharp contrast, the activity and infectiousness of beneficial entomopathogenic nematodes (EPNs) enhanced markedly under low root-exudate concentrations. Dual bioactivity in the rhizosphere was also observed for benzoxazinoids, a class of phytochemicals detected in maize root exudates. Plant-beneficial *Pseudomonas putida* was found to be recruited in response to exudation of a benzoxazinoid metabolite from maize roots during relatively young and vulnerable growth stages (Neal et al. 2012). The root colonization stimulated jasmonic acid-dependent defence pathways in maize entailing a beneficial systemic defence priming in the plant (Neal and Ton 2013). Conversely, benzoxazinoids were previously shown to exert antimicrobial and insecticidal activities and function in direct above- and belowground plant defence against pests and diseases (Niemeyer 2009; Park et al. 2004; Ahmad et al. 2011). Hence, released benzoxazinoids provide coupled profitable service for the plant by attracting beneficial microbes (indirect plant defence) and repelling pathogenic organisms in the maize rhizosphere (direct plant defence). Similarly, dimethyl disulfide emitted from cabbage (*Brassica napus*) roots invested by the cabbage root fly *Delia radicum* showed multiple defence bioactivities, the inhibition of oviposition by cabbage root fly females and the attraction of natural enemies of *D. radicum* (Ferry et al. 2007, 2009). In summary, root exudates with directed dual functions that complement each other enhance the efficiency of belowground plant protection by broadening the spectrum of defence modes and lowering carbon costs for the plant.

5 Summary

Interactions between plants and other organisms are as fascinating as they are complex. Plants can, for instance, communicate with arbuscular mycorrhizal fungi to initiate a mutually beneficial symbiosis. However, not all organisms that plants are exposed to have neutral or even advantageous impacts. Negative interactions and defence strategies against antagonistic organisms are an intensively investigated field of biology. Previously, researchers focused on interactions and processes that appear in the visible, more easily accessible half of the plant, the aerial part. However, since tremendous yield losses are caused by root feeding and infection, it is equally crucial to study plant defence mechanisms belowground.

Root exudates in the rhizosphere serve as chemical mediators of positive interactions between plants and soilborne organisms and as defence compounds in negative interactions. During plant attack root exudates are engaged in two types of defence traits, the direct and the indirect defence. Root exudates with direct defence properties act repelling, inhibiting, or killing on plant aggressors such as pathogens and feeders. In contrast, root exudates incorporated in indirect plant defence initiate the interaction with beneficial organisms that counteract aggressors. The chemical nature and mode of action of various compounds involved in direct and indirect defence have been progressively elucidated in the past years. Interestingly, several compounds were found to exhibit multiple bioactivities in the rhizosphere and influence organisms differently. In other words, a single phytochemical might act synergistically in direct and indirect plant defence. Nevertheless, another compound might recruit beneficial and detrimental organisms. Therefore, it is of importance to carefully assess the targets and effects of root exudates on multitrophic levels. In addition to the discovery of various root-secreted defence compounds and their role in the rhizosphere, the understanding of the stimulation and regulation of root exudation has advanced dramatically. Root exudation is a dynamic and bidirectional process: root exudates shape the soil inhabitants and rhizosphere members modulate the root-exudate ensemble. Besides the presence of soilborne organisms, several other exogenous as well as endogenous factors can rapidly and precisely adjust the nature of root-secreted phytochemicals. On the molecular level, transporter proteins have been shown to modulate rhizosecretion processes in a complex manner that goes beyond a role as pure substrate carriers.

Consequently, also the stimuli and regulatory mechanisms that modify the quality and quantity of the root-exudate cocktail require thorough investigation.

Taken together, root exudates impact the rhizosphere inhabitants markedly. Accordingly, they are a powerful tool that can be exploited to enhance natural defence properties of plants. Deepening our knowledge of the targets and effects of root exudates, as well as the regulation of root secretion processes, will unravel the path for more efficient disease management in the rhizosphere.

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